

The University of Chicago

THE USE OF IODINE AND OTHER
MODIFICATIONS IN THE VAN
SLYKE MANOMETRIC AMINO
NITROGEN METHOD

A PART OF A DISSERTATION SUBMITTED TO
THE FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY
AND PHARMACOLOGY

1933

By
AARON BAKER KENDRICK

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

REPRINTED FROM
THE JOURNAL OF BIOLOGICAL CHEMISTRY
VOL. 117, No. 1, JANUARY, 1937

The University of Chicago

THE USE OF IODINE AND OTHER
MODIFICATIONS IN THE VAN
SLYKE MANOMETRIC AMINO
NITROGEN METHOD

A PART OF A DISSERTATION SUBMITTED TO
THE FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY
AND PHARMACOLOGY

1933

By
AARON BAKER KENDRICK

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

REPRINTED FROM
THE JOURNAL OF BIOLOGICAL CHEMISTRY
VOL. 117, No. 1, JANUARY, 1937



THE USE OF IODINE AND OTHER MODIFICATIONS IN THE VAN SLYKE MANOMETRIC AMINO NITROGEN METHOD

By AARON BAKER KENDRICK

(From the Department of Biochemistry of the University of Chicago, Chicago)

(Received for publication, July 16, 1936)

In Van Slyke's original paper (1) on the nitrous acid method for determining amino nitrogen it was shown that cystine and glycine react peculiarly, in that they evolve, under the conditions of the determination, 6 to 8 per cent more N_2 than the theoretical amount. Other naturally occurring amino acids were found to react quantitatively with their α -amino groups, and with no other groups except the ω group of lysine. Glycyl peptides (1), glutathione (2), and amino purines (3) give high results, as do cystine and glycine.

In this paper a method is described involving the use of potassium iodide in the reaction mixture, by which theoretical results are obtained with cystine and glycine, as well as with leucine, glutamic acid, and alanine. A slight modification in the construction and use of the Hempel pipette for the manometric procedure is described, which we feel makes the manipulation more reliable.

Studies were also carried out to determine the feasibility and accuracy of methods in which the entire reaction, the absorption as well as the liberation of nitric oxide, could be carried out in the reaction chamber, and the use of the Hempel pipette avoided. Van Slyke's suggestion ((4) p. 427) involving the use of the Harington-Van Slyke (5) chamber and the absorption of the nitric oxide by permanganate, added to the chamber, was found to provide a convenient, reliable, and accurate method with several slight changes. We prefer to use N NaOH to wash the chamber instead of $5 N$, and we use a phosphate-nitrate-permanganate saturated

with air instead of the gas-free sodium hydroxide-permanganate suggested by Van Slyke.¹

The method with the Harington-Van Slyke chamber is the authors' method of choice. The Hempel pipette methods are reliable and practically as convenient, but their successful use requires a little more experience and attention to detail.

Any of the above methods may be used with or without KI and the effect of the iodide is invariably the same, to make the values for cystine² and glycine agree with the theoretical. Tryptophane is one amino acid yielding non-theoretical values, 40 to 60 per cent, when KI is used. Leucine, alanine, glutamic acid, lysine, histidine, and tyrosine yield identical values with or without KI.

The effectiveness of different amounts of KI was studied. 1 cc. of 1 per cent KI, with 5 cc. of amine in water, 1 cc. of glacial acetic acid, and 2 cc. of saturated NaNO_2 , is the least amount which will give theoretical values with cystine and glycine (with cystine, 1 cc. of 0.2 per cent KI yields 104 per cent, 1 cc. of 0.02 per cent KI yields 124 per cent of the theoretical) and 1 cc. of 2 per cent KI, the

¹ We have also developed a method for carrying out the entire reaction in the chamber of Van Slyke and Neill without the Hempel pipette. The reaction mixture of amine and nitrous acid, after liberation of the nitric oxide and nitrogen, is made alkaline in the presence of hypobromite (the hypobromite prevents formation of N_2O and N_2 by action of strong alkali on NO (6, 7)) and is then treated with an excess of oxygen, which converts the nitric oxide into nitrogen dioxide, which in turn is quantitatively absorbed by alkali. The excess oxygen is absorbed by hydrosulfite, leaving nitrogen as the residual gas.

This oxygen-hydrosulfite method yields higher than theoretical values with tyrosine (150 per cent) and tryptophane (127 per cent) owing apparently to substitution of the nitroso group in the aromatic ring and the subsequent reduction of this to nitrogen by the hydrosulfite.

Although this method is reliable with pure aliphatic amino acids, it is not recommended. Beside its inapplicability to the analysis of aromatic amino acids, it is time-consuming and the urea and ammonia corrections are large and variable, and it cannot thus be used for the direct analysis of blood filtrates.

² By Van Slyke's manometric method (without KI) we find cystine yields about 140 per cent of theoretical N_2 , while Van Slyke by his original method (1) reports about 108 per cent. This discrepancy is doubtless due to the differences in the conditions of the two methods. The concentration of reagents is practically the same in both; but mercury is in contact with them in the manometric method.

amount prescribed in the procedure below, provides a definite excess. This is about the minimal amount which causes precipitation of free iodine when mixed with nitrous acid under the conditions of this analysis. Greater amounts of KI, up to 1 cc. of 40 per cent, also give theoretical values with cystine and glycine. They are, however, undesirable, for aside from causing wasteful precipitation of iodine and increasing greatly the amount of nitric oxide evolved, with certain amino acids (and urea) the rate of nitrogen formation is greatly decreased, so that with alanine, for example, 10 minutes must be allowed at 22° in order to get a theoretical nitrogen value. With 1 cc. of 2 per cent KI even alanine, the most slowly reacting amino acid in our series, gives theoretical values in the time intervals specified by Van Slyke ((4) p. 440) (5 minutes at 17.5°, 3 minutes at 25°, etc.). Urea, under these conditions, yields 5.5 per cent of its nitrogen and this is the urea correction which must be applied when blood filtrates are analyzed by the KI methods given.

In the presence of larger amounts of KI, urea liberates less nitrogen; with 1 cc. of 20 per cent KI, only 1 per cent in 3 minutes at 25° (Van Slyke's time and temperature). This is a negligible urea correction for normal blood filtrates, but this amount of KI with these time intervals may not be used with blood filtrates, since certain amino acids yield nitrogen incompletely under these conditions: alanine, 93 per cent, and leucine, 98 per cent. When with 1 cc. of 20 per cent KI the time is increased to 7.5 minutes at 25°, so that alanine and leucine give theoretical values, the urea correction is increased to 3.5 per cent. It is possible to make accurate blood filtrate amino nitrogen determinations with 1 cc. of 20 per cent KI, and we have determined in detail the times and temperatures which will give nitrogen equal to 3.5 per cent of the urea. These are 17.8 minutes at 15°, 11.5 minutes at 20°, 7.5 minutes at 25°, 4.8 minutes at 30°. We prefer, however, for blood filtrates as well as for all other amino nitrogen work with KI, to use Van Slyke's times and temperatures ((4) p. 440) with 1 cc. of 2 per cent KI in 97 per cent acetic acid, under which conditions urea yields 5.5 per cent of its nitrogen.

In studying the mechanism of action of the KI, it was found that an equimolecular amount of iodine has the same effect as iodide in giving theoretical values with cystine and glycine. Since the

iodide, when mixed with nitrous acid, is immediately oxidized to iodine, one may conclude that it is the oxidizing action of the iodine, rather than the reducing action of the iodide, which is the effective agent. This is supported by the observation that if in place of 1 cc. of 20 per cent (1.2 N) KI, 1 cc. of 0.6 N KI and 0.6 N $\text{Na}_2\text{S}_2\text{O}_3$ is used, under which condition practically no iodine is liberated by the nitrous acid, the values with cystine are about 140 per cent, the same as by Van Slyke's method in which no iodide is used. Since the ferric-ferrous system has approximately the same oxidation-reduction potential as the iodine-iodide system, it was thought that these ions might be of value here. However, ferric or ferrous chlorides, in a wide range of concentration, from 1 cc. of 0.12 M to 1 cc. of 1.2 M, have no effect on the N_2 values obtained from cystine with nitrous acid, the values being about 140 per cent, just as when no addition is made. We have also observed that when cystine is treated with nitrous acid in the presence of iodide (or iodine), sulfate is formed, just as is the case in the absence of iodide, the evidence being the white precipitate with barium chloride (8, 9).

It is generally agreed (1, 8, 9) that there are present in cystine and glycine some "abnormal" reducing groups which are not present in the other amino acids. In cystine, this abnormal group is doubtless the sulfide sulfur. In the absence of iodine this abnormal group reduces the nitrous acid with the formation of extra nitrogen. We believe that in the presence of suitable amounts of iodine, this abnormal group is oxidized before it can react appreciably with the nitrous acid, and thus theoretical values are obtained.

Comparative studies of the same blood filtrates by different methods (Table I) show that the KI methods yield consistently lower results, by 8 to 15 per cent, than do Van Slyke's method and the other non-KI methods. This agrees with the comparison made by Van Slyke and Kirk ((10) p. 675), on analysis of amino nitrogen of blood by different methods, where it is found that the values obtained by Van Slyke's manometric method are consistently higher, by 5 to 14 per cent, than the values obtained by the formol titration. This is what we should expect if blood filtrates contain significant amounts of cystine, glycine, and other compounds known to yield greater than theoretical values by Van

Slyke's amino nitrogen methods. The explanation may lie in part in such compounds as tryptophane, which show too low results by the KI methods. A similar comparison made with the use of 1 cc. of 20 per cent KI, in which the urea corrections are 3.5 per cent (instead of 1 cc. of 2 per cent KI), has shown essentially the same difference between the KI and the non-KI methods, from 8 to 14 per cent. More detailed study of the analysis of

TABLE I

Effect of KI upon Amino Nitrogen Values of Blood Filtrates

Method A is Van Slyke's manometric method. In Method B the Harington-Van Slyke chamber is used, and the NO is absorbed directly in the chamber with KMnO_4 . Methods AA and BB are the same as Methods A and B respectively, except that KI is added.

	Amino N per 100 cc. blood				Per cent by which Method A or B exceeds AA or BB	Urea N per 100 cc. blood
	Method A	Method B	Method AA	Method BB		
	mg.	mg.	mg.	mg.		mg.
Human, normal.....		9.86		8.91	10.7	15.0
“ “		9.13		8.24	10.8	12.2
“ “		10.08		9.12	10.5	9.03
“ uremic.....	8.41	8.25	7.75	7.36	10.3	45.9
“ “		8.29		7.20	15.1	53.2
Dog 7.....	10.93	10.68	9.93	9.49	11.2	16.8
“ 9.....	13.49	13.67	12.50	12.45	9.0	24.2
“ 10.....	12.99	12.55	11.70	11.47	10.2	16.5
“ 11.....		11.49	10.69	10.61	7.8	14.3
“ 13.....	14.15	13.79	12.19	12.04	15.3	12.0

All these amino nitrogen figures are corrected for urea nitrogen, Methods A and B by 7 per cent, Methods AA and BB by 5.5 per cent.

blood filtrates by these methods will be reported in a subsequent paper.

Studies on hair hydrolysates have shown that the amino nitrogen values without KI are from 8 to 11 per cent higher, and with gelatin hydrolysates (in which about 25 per cent of the nitrogen is in glycine) from 5 to 10 per cent higher than the values with KI.

In the following pages three modified methods are described: (a) the original Van Slyke method plus KI, (b) the method with the Harington-Van Slyke chamber and KI, (c) the method with

the Harington-Van Slyke chamber without KI. Finally there is described a modified Hempel pipette.

Original Van Slyke Method Plus KI

This procedure is exactly the same as Van Slyke's manometric method (4), with the following modifications.

In place of glacial acetic acid as a reagent, a solution containing 2 per cent KI in acetic acid is used. 2 gm. of KI are dissolved in 2 cc. of water and made up to 100 cc. with glacial acetic acid.

After the nitrous acid reaction mixture has been expelled from the chamber, the chamber is washed once with 5 per cent sodium thiosulfate to remove iodine, before the usual two washings with water.

Method with Harington-Van Slyke Chamber and KI

Reagents—The saturated sodium nitrite and 2 per cent KI in acetic acid are identical with those described above.

Permanganate-phosphate-nitrate solution. To 5 gm. of KMnO_4 , 18 gm. of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ (or 7 gm. of anhydrous Na_2HPO_4), and 60 gm. of NaNO_3 add 100 cc. of water and warm with stirring until all is in solution. This solution should be kept above 20° , otherwise some of the salts will crystallize out. The 5 per cent KMnO_4 in 10 per cent NaOH described by Van Slyke (4) may be used here with the same accuracy, but we prefer the phosphate-nitrate-permanganate because there is less fouling of the mercury by the reagent. A phosphate-permanganate solution without nitrate may also be used, but the blanks are much higher, owing to the larger amount of dissolved gas in this reagent.

Gas-free N NaOH. 4 per cent NaOH is made gas-free by boiling or by repeatedly evacuating and expelling the extracted gases in the Van Slyke-Neill gas apparatus, after which it is stored in a gas-free reagent container over mercury.

A convenient form of gas-free reagent container, which we have been using for several years, is described in Fig. 1 (11). It is somewhat more easily made than those described by Guest and Holmes (12). It may be supported when not in use by a small conveniently portable wooden rack. The delivery capillary is so made that it fits into the bottom of the cup of the Van Slyke-Neill reaction chamber; and liquid may be caused to pass in either direction without exposure to air.

Procedure

The first two steps in this analysis, namely the removal of air from the mixture of amine, acetic acid, and iodide and the decomposition of the amino groups, are the same as in the above method.

Removal of Nitrous Acid Solution and Washing of Chamber— After the time to be allowed for the decomposition of the amino groups is complete, the leveling bulb is held in a position about 40 cm. below the 50 cc. mark. With the leveling bulb stop-cock

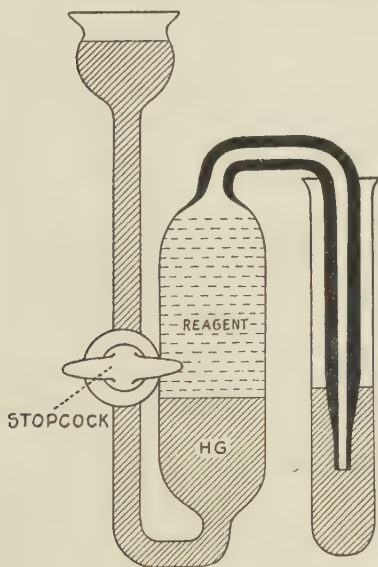


FIG. 1

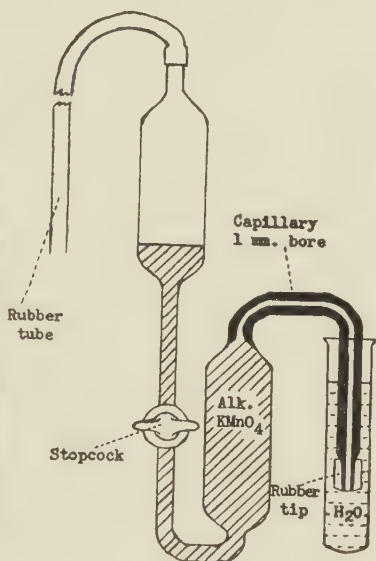


FIG. 2

FIG. 1. Pipette for storing gas-free reagents over mercury.

FIG. 2. Modified Hempel pipette.

open, the flow of mercury from the chamber into the trap is controlled with the stop-cock at the bottom of the Harington-Van Slyke chamber, until all except about 0.1 cc. of liquid has left the chamber. The liquid is then expelled from the trap.

Care is taken that the cup is free from water. With the tip of the N NaOH gas-free reagent container near the bottom of the cup, the stop-cock of the container is opened so that the liquid flows into the cup at a rate of about 1 cc. in 2 seconds. When the cup is nearly filled (and while the liquid is still flowing from the con-

tainer) about 2 cc. of the solution are run quickly into the chamber by controlling the reaction chamber stop-cock. When the cup fills again, the reagent container is removed.

The 2 cc. of washing solution have now drained to the bottom of the chamber and are promptly removed into the trap. About 4 cc. of additional liquid are admitted from the cup, leaving at least 1 cc. in the cup as a protective layer against absorption of air.

The stop-cock is sealed with mercury and the chamber is shaken for about 15 seconds. This shaking should be so vigorous that the liquid splashes effectively against the walls of the reaction chamber, and all of the precipitated iodine is removed. During this shaking with the leveling-bulb stop-cock open, and the stop-cock at the bottom of the reaction chamber closed, the leveling bulb is lowered until the liquid in the trap from the previous washing is drawn down to the bottom of the trap so that all of the precipitated iodine on the walls of the trap is effectively removed. The motor is stopped and the liquid in the trap, together with any supernatant gas, is expelled. The liquid in the chamber is then drawn into the trap, leaving about 0.1 cc. of liquid in the chamber, so as surely not to draw any gas from the chamber. The liquid in the trap is drawn down to the bottom of the trap and then expelled. The stop-cock at the bottom of the chamber is finally left open to the chamber.

Absorption of Nitric Oxide with $KMnO_4$ —6 cc. (or more) of the phosphate-nitrate-permanganate solution are introduced into the cup and 3 cc. are admitted at once into the chamber. The chamber is shaken slowly with the motor for about 10 seconds, and as the gas is absorbed, the mercury rises in the chamber. An additional cc. of $KMnO_4$ is added and the chamber is shaken for another 10 seconds, while the volume of the gas is so reduced that the aqueous meniscus enters the constricted portion of the chamber near the 2 cc. mark. The remaining 2 cc. of $KMnO_4$ are added gradually in about 1 minute in about four 0.5 cc. instalments. Agitation is obtained by raising the leveling bulb gradually about 15 cm. above the lower ring support, and then letting it down suddenly to the lower ring support. Care must be taken not to lower the leveling bulb below the lower ring support, so as not to draw dissolved air out of the permanganate solution; and not to raise the leveling bulb above the level of the mercury in the

chamber, so as not to lose gas through the stop-cock at the top of the chamber. The rate of addition of the permanganate and the method of agitation may be varied greatly without sacrificing accuracy. It is important to make analyses and blanks in the same manner. If the absorption of nitric oxide is not complete after 6 cc. of KMnO_4 have been added, one should add 1 or 2 cc. of additional permanganate from the cup. Unless unusually large amounts of NO are present (more than 30 cc. at 1 atmosphere), 6 cc. of KMnO_4 are ample to effect complete absorption.

The cup is washed several times with water until free from permanganate color, and about 2.5 cc. of gas-free N NaOH are introduced into the cup, 2 cc. of which are admitted to the chamber. The stop-cock is sealed with mercury.

Measuring the Nitrogen Gas—After $\frac{1}{2}$ minute (to allow for complete absorption of CO_2 and N_2O) the aqueous meniscus is brought exactly to either the 0.5 or the 2.0 cc. mark and the manometer reading, p_1 , together with the temperature, is recorded. It is important that this step be carried out with a minimum of agitation in order that as little as possible of the dissolved air will be extracted from the permanganate solution.

The gas is expelled with minimal loss of liquid (as much as 0.2 cc. of liquid loss does no harm). After sealing the stop-cock with mercury, the aqueous meniscus is again set at the 0.5 or 2.0 cc. mark and the manometer reading, p_0 , is recorded.

For washing the chamber between analyses, the use of dilute nitric acid or NaNO_2 and acetic acid is very effective. The nitrous acid must be completely removed by at least three washings with water before the next amine sample is introduced.

After many analyses a yellow solid sometimes collects in the trap below the reaction chamber, which is not removed by ordinary washing of the chamber. The following washing method has been found effective in removing this solid.

After rinsing the chamber with NaNO_2 and acetic acid, followed by water, add about 5 cc. of saturated $\text{Na}_2\text{S}_2\text{O}_3$ solution to the chamber, followed by about 50 cc. of water. With both stop-cocks of the chamber and the stop-cock to the leveling bulb open, the leveling bulb is gradually and *cautiously* lowered so that the water in the chamber flows into the glass tubing below the reaction chamber, until it reaches the 4-way junction above the leveling bulb

stop-cock, when the leveling bulb stop-cock is closed. Care must be taken to avoid lowering the leveling bulb so rapidly that the water enters the manometer tube. A suction tube leading to the waste bottle is attached to the glass tubing immediately above the stop-cock which is above the 4-way junction. This stop-cock is then gradually and cautiously opened until the water meniscus in the chamber falls to the 50 cc. mark. (Care must be taken not to open this stop-cock so suddenly that a considerable amount of mercury is drawn out of the manometer, so that water later would be sucked into the manometer.) The leveling bulb is placed in the upper ring support and the stop-cock leading to the leveling bulb is gradually opened, until the mercury has flowed into the reaction chamber to about the 50 cc. mark, and continuity to the mercury in the manometer is established. Then the stop-cock above the 4-way junction is opened until the water is completely displaced with mercury. The water is expelled from the reaction chamber and the chamber is washed twice with water.

The blank analysis of reagents and calculations are the same as the description given by Van Slyke ((4) pp. 434-437).

Method with Harington-Van Slyke Chamber without KI

The procedure is the same as that with KI, except that glacial acetic acid without KI is used.

Construction and Use of Modified Hempel Pipette

In our experience with Van Slyke's form of Hempel pipette, small amounts of gas occasionally become trapped in the capillary adjacent to the stop-cock. This occurs usually when grease collects in and around the bore of the stop-cock and especially in old pipettes when after extensive use small grooves or nicks appear on the core of the stop-cock. This difficulty can be entirely avoided by a pipette in which there is no stop-cock in the delivery capillary, and in which the stop-cock controlling the flow of gas is in the tube between the two reservoirs (see Fig. 2). This pipette is also less expensive and in our experience less apt to be broken. The details in the use of this pipette will now be described.

Transfer of NO + N₂ Gas Mixture to Permanganate Pipette and Absorption of NO After the amine-nitrous acid reaction is complete, the stop-cock leading to the leveling bulb is opened and the

leveling bulb is allowed to remain in the lower ring support. About 1 cc. of gas-free water³ is introduced into the cup above the chamber. By momentary opening of the reaction chamber stop-cock to the cup, the mercury in the capillaries is displaced with gas-free water. (If this mercury were not removed, it would later be driven into the KMnO_4 pipette, which is to be avoided.)

The tip of the pipette (see Fig. 2) is introduced into the cup, and by opening the pipette stop-cock about 6 cc. of KMnO_4 solution are allowed to flow from the pipette into the cup while the tip of the pipette is held below the liquid level. The pipette tip is then pressed and held firmly into the bottom of the cup, while its stop-cock is allowed to remain open wide. The leveling bulb of the gas apparatus is placed in the upper ring support, and after the gas in the chamber is under greater than atmospheric pressure, the leveling bulb stop-cock is closed. The reaction chamber stop-cock is then opened wide, and by controlling the leveling bulb stop-cock, the $\text{N}_2 + \text{NO}$ mixture is caused to flow into the pipette. As soon as the first visible portion of nitrous acid solution enters the pipette capillary, the leveling bulb stop-cock is closed, and the reaction chamber stop-cock and the pipette stop-cock are also closed. The leveling bulb is placed in the lower ring support. In blood filtrate analyses it is important that not more than 0.03 cc. of the iodine-nitrous acid solution enter the Hempel pipette capillary.

While it is held with firm pressure in the bottom of the cup, the pipette is shaken with a horizontal back and forth rotation for about 5 seconds (not longer), which promptly creates a vacuum in the pipette. The shaking is stopped and the pipette stop-cock is opened until the KMnO_4 solution from the pipette reservoir has restored the gas pressure. The pipette stop-cock is closed and a second and third shaking of 10 seconds each and restoration of pressure are effected. After a third (or fourth) 10 second shaking, when a small amount of gas still remains to be absorbed, the gas pressure is restored by lifting the pipette tip from the bottom of the cup, so that the pipette capillary is filled with KMnO_4 solution from the cup. Here it is important to watch the level of the permanganate in the cup, and if it falls to as low as 2 cm. from the

³ Distilled water is boiled and drawn while hot into a gas-free reagent container over mercury (see Fig. 1), where it is stored.

bottom of the cup, the flow of liquid is stopped by pressing the tip again into the bottom of the cup; in this case the pressure is finally restored by opening the pipette stop-cock. Usually after the fourth shaking the volume of gas remaining to be absorbed is less than 3 or 4 cc., and after the pipette tip is once lifted from the bottom of the cup at this stage, it is not necessary to press it down again. With the tip lifted from the bottom of the cup, and while still immersed in the KMnO_4 solution in the cup, the pipette is shaken a final 30 seconds to insure complete absorption of NO . The pipette is then removed from the cup and supported with the tip immersed in a test-tube containing water.

In this manipulation the duration of the first and second shakings should not be longer than 5 and 10 seconds respectively in order that the vacuum will not draw appreciable amounts of dissolved air from the permanganate solution. If the gas is all absorbed before the pipette tip is lifted (as may inadvertently happen when one opens the stop-cock instead of lifting the pipette after the fourth shaking), one fills the pipette capillary with KMnO_4 solution, by applying gentle suction with the mouth through a rubber tube attached to the open end of the pipette reservoir, and momentary opening of the pipette stop-cock, while the lifted pipette tip is immersed in the KMnO_4 solution in the cup.

Any one of several other manipulations may be used to effect the absorption of the NO in the pipette; for example, the pipette stop-cock may be left open throughout the absorption, and the pipette capillary finally filled with KMnO_4 solution from the cup by suction applied to the open end of the pipette reservoir.

Return of Purified N_2 to Manometric Chamber—After the chamber is washed and charged with about 10 cc. of gas-free water, and 1 cc. of this water is returned to the cup, the cup is nearly filled with ordinary water. With the leveling bulb stop-cock open, the leveling bulb is raised to the upper ring support until there is no evacuated space in the manometer. The leveling bulb stop-cock is closed and the leveling bulb placed in the lower ring support.

The tip of the Hempel pipette is introduced below the surface of the water in the cup and held there, while the pipette stop-cock is carefully opened until most of the permanganate has flowed out of the pipette capillary and the permanganate- N_2 meniscus is

about 2 cm. from the tip. The tip of the pipette is pressed and held firmly in the bottom of the cup and the pipette stop-cock and also the reaction chamber stop-cock are opened wide. By controlling the leveling bulb stop-cock, the gas in the pipette is caused to flow into the reaction chamber and, as soon as the first permanganate following the gas is seen to enter the capillaries of the reaction chamber stop-cock, the leveling bulb stop-cock is closed. The other two stop-cocks are then closed and the pipette is removed.

The cup is washed until free from permanganate color and about 0.2 cc. of water is drawn into the chamber from the cup in order to displace the permanganate from the capillaries at the base of the cup and of the reaction chamber stop-cock. The stop-cock is then sealed with mercury. We have found it desirable to use a minimal amount of mercury here for, as the mercury droplets fall through the gas and water phases, they carry bubbles of gas down to the mercury-water surface, which at times has a significant lowering effect on the subsequent pressure reading. This is all the more important since in setting the meniscus in preparation for this reading, all agitation must be avoided in order to prevent the solution of the N_2 in the water. The washing with about 0.2 cc. of water immediately before sealing the stop-cock with mercury renders the use of all except minimal quantities of mercury unnecessary.

SUMMARY

By the use of potassium iodide in the reaction mixture, Van Slyke's manometric amino nitrogen method has been modified to yield theoretical values with cystine and glycine, as well as with other common amino acids. A simplified form of Hempel pipette is described, which we feel makes the method more reliable.

A method is described in which the entire analysis is carried out in the Harington-Van Slyke chamber, and which thus avoids the use of the Hempel pipette. This method is recommended as the most convenient and uniformly reliable of the manometric amino nitrogen methods.

With blood filtrates (after correction for urea) the KI methods yield from 8 to 15 per cent less N_2 than Van Slyke's method, indicating that blood probably contains significant amounts of sub-

stances like cystine and glycine, which yield too high values for amino nitrogen by Van Slyke's method.

BIBLIOGRAPHY

1. Van Slyke, D. D., *J. Biol. Chem.*, **9**, 185 (1911).
2. Hopkins, F. G., *J. Biol. Chem.*, **84**, 271 (1929).
3. Wilson, D. W., *J. Biol. Chem.*, **56**, 183 (1923).
4. Van Slyke, D. D., *J. Biol. Chem.*, **83**, 425 (1929).
5. Harington, C. R., and Van Slyke, D. D., *J. Biol. Chem.*, **61**, 575 (1924).
6. Gay-Lussac, J. L., in Mellor, J. W., *A comprehensive treatise on inorganic and theoretical chemistry*, New York, **8**, 432 (1928).
Russell, W. J., and Lapraik, W., *J. Chem. Soc.*, **32**, 35 (1877).
7. Barr, G., *J. Chem. Soc.*, **125**, 961 (1924).
8. Schmidt, C. L. A., *J. Biol. Chem.*, **82**, 587 (1929).
9. Lough, S. A., and Lewis, H. B., *J. Biol. Chem.*, **104**, 601 (1934).
10. Van Slyke, D. D., and Kirk, E., *J. Biol. Chem.*, **102**, 651 (1933).
11. Oesting, R. B., *Physiol. Zool.*, **7**, 543 (1934).
12. Guest, G. M., and Holmes, F. E., *J. Biol. Chem.*, **110**, 781 (1935).
Holmes, F. E., *J. Biol. Chem.*, **113**, 411 (1936).

WAVERLY PRESS, INC.
BALTIMORE, MD.